

Apr. 29, 1948.

	Dlu	Mal	Lac	Gal	Glu	Megal.	T1
182	++	+	-	+	+±		S
185	++	-	-	-	-		S
187	<del>thin</del>	-	-	÷	÷		S
188	-	-	-	++	+		S
189	-	-	-	-P	+		*
218	+	+	++	+	+		S
239	-	-	-	-P	-P		S
243	+	-	-	÷	÷		S
245	-	-	+	++	++		S
- 253	+	+	+	+	±		S
319	-	-	-	+	+		S
321.	++	++	++	++	++		S
✓ 108	- v.pop.	-v.p.	-v.p.	++	++		S

These are suspensions from fairly old cultures.

\* v. few plaques.

B

47  
72  
74  
76  
83  
87  
~~108~~  
~~110~~  
~~112~~

S.O. 321. on glucose lac

245. on lac 1.6% gal, glucose. Single plaques!

218. pl.

182 lac for S.O.

185 lac for

108 S.O.

Eng. Phage Test

Try O.P. effects on types thin on glucose I.

Many are "thinner" on glucose than on disaccharides - e.g. 187, 218,

S.O. 249 on lactose 90%+. Purify also - for test as Lac<sub>3</sub>.

243 on lactose. All colonies are slow ++. Blood streaks is -. One (-) colony noted. Purify.

245 on lactose. - and very faint ± colonies predominate, with numerous papillae +.

S.O. - colony on lactose EMBS: all - colonies.

Test:	Lac	Mal	Tac	Glu	Gal	Suc	
	108 pur	-	-	++	++	-	W108
	245Lac-	= papillae	±	+	++	-	
184, 1-3.	243Lac+	-	±	+	±	- th.	W381
	249Lac-	-	-	+	++	-	
	243Lac-	-	±	+	±	- th.	W243

Bacterial purified W108 on Lac

249 is comparable to W108 and may be Lac<sub>3</sub>-. 243Lac+ may be a segregant. Cell 243Lac- = W243 as recovered, and 249Lac+ = W381

Reconstitute all these stocks.

W185, fructoset: Colonies small & slow on glucose. 95%+. Some - noted.

Mannose All +.

Sorbitol All -

Fructose All +.

Recover

~~Fructose~~ glu- and compare with + on extended series of sugars.

Reversions of W-245

177b.

May 5 + 1948.

Stockout 177a, W-245/Mal on Mal E-1413.

Pick 14 Mal+ colonies to Lac and Blu. at 37°.

a) All 14 are Lac++ Blu-

b) 3 Mal+ colonies Lac± Blu-

1 Mal- colony Lac- Blu- apparent.

S.O. from a and b on maltose to purify. W397 + W398

Megal.

~~Megal.~~ Megal

	K-12	+++
A	W-108	+
	243	+
	260	-
	261	+
	267	++ (variable)
	269	+
	270	+
	277	-
	280	+
	284	-
	285	-
	292	-
B	297	++ var.
	298	+
	301	-
	307	++
	308	+
	312	+
	249	+
	257	-
	258	-
C	319	-
	322	+
	321	+
	120	- n +
	RS. 1	+++
	RS. 2	++#
	453	++#
	410	+++

1	112	+++	+++
2	121	++	+++
3	276	-	-
4	283	-	-
5	286	-	-
6	287	-	+++
7	313	+	-
8	316	-	-
9	317	++	++
10	122	+	- (variable)
"	132	-	-

\* 312 + 302 were found filled with water! Some?  
SO on glucose.

Glucose - mutation run

18°

April 28-30, 1948.

58-161R. 135 plates  $\times$  >100 scoreable colonies  
= ca 15,000 total.

15 tiny colonies found. None mutants.

No mutants from ca 6 other sectors.

### Formate mutation Run.

Y10. Spiked on Glucose 1%, Formate .4% EM103 and irradiated as above. 46 plates  $\times$  500/plate = 25,000 colonies.

Due to crowding it is not certain how efficient mutant recovery would be. Test some representative colonies.

Formate mutations.

180<sub>9</sub>.

May 1, 1948.

Compare - (glucose EMB+) and + (-) colonies from formate-glucose EMB on  
 + (a). Formate .5% Nase & thal. 01% agar  
 (b) Formate - phosphate Nase gas tubes.

EMB.	(a)	(b)	(c) EMBS form. glu.
1. 1-	++	+++	-
2. 1-	1-	-	+++
3. 1-	-	-	-
4. 1+	-	-	-
5. 1+	-	-	-
6. 2-	-	-	-
7. 2+	-	-	-
8. 3-	-	-	-
9. 3+	-	-	-
10. 4-	-	-	-
11. 5- (imp?)	-	-	-
12. 6-	-	-	-
13. 6+	-	-	-
14. 7-	-	-	-
15. 7+	-	-	-
16. 8-	-	-	-

All cultures produce voluminous gas from formate broth

1) cannot be scored due to diffusion of alkali through agar.

\* Streak out 1, 4, 6, 7, 8, 9 12, 13 + 14, 15 on glucose EMB. Indistinguishable!

Test streaks on formate glucose agar.

\* + = 1, 4, 6, 7, 8, 9, 12, 13

Transfer (6) to nutrient agar slant as W-385

For fungi

Test N-12 on:

24 hr.

48 hr.

1. EMB - 2% Na glycerophosphate + 5% H<sub>2</sub>O. Large - colonies. ✓
2. 1% Peptone acid, neutralized NaOH. N.S. Agar very soft. ✓
3. Hydrolyzed casein (HC) agar. Moderate colonies.
4. HC - succinate - Chlorophenolendolphenol. Moderate colonies.  
Agar was decolorized after autoclaving. Shows diffuse recoloration around colony groups.  
entire plate decolorized  
colorless ground surface  
colonies tan or yellowish
5. HC + succinate + Cl<sup>-</sup> " v. slight lightening around colony mass  
colorless ground surface  
colonies tan or yellowish  
+ C.C. in U.V.
6. HC - NaCl. No growth. Spontaneous coloration in agar overlying lit.
7. HC - Indigo sulfate .01% Decolorized on autoclaving + agar  
+ succ. } Moderate colonies; no recoloration.  
- succ. }
8. HC - starch, Iodine.  
+ succ. } Colored discharged on precipitation (I<sub>2</sub>) reduced.  
- succ. } large, slightly brownish + tan + colonies.
9. Sorbitol 1% ++ Not quite so intense + as sucrose but unquestionably strong +.
10. Sorb. 5% + Galactose 5% ++ No inhibition
11. (fructose)
12. Galactose .5%. ++ - ✓

K-12"; W-145; growth on synth. medium.

183

April 30, 1948.

Dose W-145 lightly into T(m) TLB, BM + .1%

	24h.	72h.
1. Glucamate	-	+++
2. Glucose	+++	++
3. Lactose	±	++
4. Maltose	+	+±

Inoculate further and examine for  
esp. reversions. S.O. P3 on homologous  
medium.

58-161 into.

1. Na glyceophosphate .5H <sub>2</sub> O	0.2%	24h. +++	S.O. Gp plate
2. Pectic acid; neutr. NaOH.		Faint ± on EMB	
EMB.	58-161 -	72h. +	
	Y10 -	+	+ faint growth in synth.

P3.

↓ S.O. 1, 3 and 4 on homologous EMB agar.

1. No acid production; colonies very substantial

3. Numerous + colonies. Pick to gna EMB

4. Maltose - all -

5. 14 colonies all -. Purify on lactose EMB.

W-391

April 29, 1948.

110 1 drop, etc. (Haworth lamp 5 secs.) on glucose EMB.

Most of 52 plates were mainly contaminated.

Select some likely colonies from 20 best cont. plates; ca 500 scoreable colonies

3 Glucose - streak across T1. All V<sup>s</sup>.

= 19,000.

	Glu	Gal	Tae	Mal	Glu
1. W-382	- *	+++	+++	+ <sup>pp</sup>	++
2. W-383	-	±	±	-	++
3. W-384	-	++	-	-	++

-382. Why papillae only on maltose? This appears to be the desired Glucose-specific mutant, for crosses with Gal -.

\* produces acid strongly when left out at room temperature 2-3 hours!  
(compare 340).

~~Streak out 382 and 340 on each of two glucose plates. Incubate overnight at 37°.~~ See 185

5-3-42

Strains out to form colonies of: (on EMBA 1%):

	Rhamnose	Glucose	Sorbitol	Fructose	Mannitol	Mannose	Galactose	D-Glucitol	Mucic acid	Xylose	N-Acetylglucosamine	
1. 254 *	-	++	++	+	v	++	++	++	v	+	-	-
2. 108	-	++	-	-	-	-	-	v	-	+	-	
3. 185 b + mch inb	-	++	-	inb	-	++	-	-	-	(1 <sup>nh</sup> )	-	
4. 185 b -	+	-	-	-	A6P.	-	P	-	P	-	A6P.	
5. 249	-	+	-	*	-	-	-	-	±	++	A6P.	
6. 351	-	++	++	v	++	v	++	v	++	v	A6P.	
7. 361	-	++	++	v	++	v	++	v	+	v	v	
8. 58-161	+	++	++	v	++	v	++	v	++	v	v	
Y10 -	<i>mannitol</i>											
	A6 some v x											

p = papillar, presumably mucoid.

\* S.C. on homologous medium, A6

Lac<sub>3</sub> Crosses

May 4, 1948.

Cross the following on EMS-Lac-B<sub>1</sub>.

1. W-108 x W-249 (A conc. susp) T-L-B<sub>1</sub>-Lac<sub>3</sub> x B-M-Lac<sub>X</sub>  
 2. W-108 x Y-40 x B-M V<sub>1</sub><sup>r</sup> r  
 3. W-249 x Y-46 x T-L-B<sub>1</sub>-V<sub>1</sub>

P7.

① Yield very poor.

By plate.

	+	-	to retest
	0	1	
	0	1	
	0	0	
	0	0	
	0	1	
A.	0	1	
A.	0	5	
A.	0	3	
A	0	4	
	0	2	
	0	0	
	0	3	
	0	1	
A	0	2	
	0	1	
	0	0	
	0	2	
	0	0	
A	0	3	
A	0	4	
	<hr/>		
	0	38	1

After several days incubation, some lac+ 's came up. Since these may represent crossovers, do not use these plates.

(2)

	+	-
2	31	
1	25	
6	34	
2	52	
2	30	
4	50	
0	32	

$$\begin{array}{r} 17 \quad 25 \bar{4} \\ \hline 281 \end{array} = 6.7\% \text{ Lac}_3 +.$$

$T-L-B_1-Lac_3-B+M+$  } x  
 $T+L+B_1+Lac_3+B-M-$  }

$Lac_3$  is fairly closely linked to  $B14$ . (very near  $Lac_2$ )

Phage tests (on glucose plates).

$Lac+$ :	$6^R$	$2^S$	$  8$	All blue +
$Lac-$ :	$48^R$	$13^S$	$  61$	All blue -
$Lac-$ :	$51$	$12$	$  63$	$] \% V^R = 80\%$
	99	25	124.	

(3). Very poor yield on a rather dense background.

0	1
0	0
0	0
2	0
0	0
1	0
	;
4.	3

May 3, 1948.

$$100 \text{ plates DluEMB} \times 250/\text{plate} = 25,000.$$

17 tiny colonies streaked whole on glucose

3 - (1-3)

14 other possibles S.O. on glucose.

1.	4	0	mucoid
2.	5	0	+
3.	6	0	+
(4)	7	0	+
(5)	8	0	-
	9	0	-
	10	0	+
	11	0	+
	12	0	+
	13	0	+
(6)	14	0	slow?
(7)	15	0	- com. cl.
	16	0	+
	17	0	+

1, 2, 4, 5 and 7 are T, S, and probably mutants.

3 is a yellow charragon { almost certainly contaminants.  
6 a pink charragon }

W-						
1.	386	-	-	+ slow	+	++
2.	387	±	±	+	±	++
4.	388	-	-	- th. - th.	+	-
5.	389	Gluc. +	+	+++	+++	+++
7.	390	Glu +	++	+++	+++	+++
	391		+++	-	±	-

→ specifically bac +

May 5, 1948.

1. 108 x 58-161      on glucose ± B,
2. 249 x 108      on glucose B,
3. 382 x 249      on glucose, lactose
4. 382 x 58-161      glucose, lactose.

P7:

1 - B<sub>1</sub>.

		+	-	
		<del>T</del>	<del>T</del>	
		19	177	
		16	133	
35		300	335	

To be properly  
carried

+ B<sub>1</sub>,

		21	163	184	
56		463	519		

Some colonies are darkened  
but probably not T

<sup>Second</sup>  
P10.) 2. Yield negligible (ca<sup><1</sup> purple)

3. (glucose) Yield negligible - all-  
lactose. All look "+" after prolonged incubation. Score on glucose, T<sub>1</sub>.

4. Glucose - measurable - no yield  
lactose - all turned +.

Tetragolium

192a

May 7, 1948.

- ①. Make up varying concentrations of triphenyl tetragolium chloride in nutrient agar and autoclave. Sterile 1100<sub>r</sub> plates.

Per ml:

- 1mg. Medium faint pink; all colonies intense disper.
- 150r Medium sl. tinged; isolated colonies deeply red with Strep. mayores.
- 50r As above. Medium less tinged
- 30r As above for isolated colonies; confluent growth colorless
- 10r Color more limited in colonies and sl. less intense.

1mg. level shows slight initial growth inhibition

Lac 3 mapping. May 10, 1948

- ① W-108 x Y40.      in lac and glu EMS (NF).  
 ② W-249 x Y46  
 ③ W-108 x W-249.

3:

	-	+
24	0	
55	0	
9	0	
10	0	
31	0	
L 67	0	
L 32	0	
L 24	0	.
L 22	0	
L 25	0	
L 11	0	
L 31	0	
L 26	0	
L 31	0	
L 41	0	
L 24	0	
L 16	0	
L 17	0	
5	0	
<hr/>		
Lac:	191	0.
Glu:	310	0

~~total 501~~ 0

SDI.

Both are probably Lac<sub>3</sub> -.

(2)

Plates v. unsatisfactory. Overgrown or noxious. Sample plates  
readable, esp. lactose.

	+	-
18	2	
2		1
16	4	
3	2	
3	0	
4	0	
4	1	
3	0	
7	0	
7	2	
	67	12
		789..

This count unsatisfactory except to indicate more + than -.

(1)

Lac.

	-	+
53	8	
45	13	
24	3	
39	10	
14	5	
44	16	
29	3	
31	4	
35	8	
42	11	
42	9	
39	8	
75	7	
	512	105
		617

all scored (-) as glucose,  
probably due to unsatisfactory  
of medium. Test by streaking  
to fresh glucose EMB.

$$= 17\% \text{ Lac+} \quad 83\% \text{ Lac-}$$

Test Lac+ on Glu, T1:

R	S
22	2
17	2
13	1

$$= 13\% \text{ B among Lac+}$$

Test Lac- on Glu, T1

Test Lac- segregants on T<sub>1</sub> (Glu or Gln or TMS!)

R	S.	
15	5	20
14	6	20
14	6	20
13	7	20
56	24	80 ✓

30%<sup>s</sup> among Lac- .

The distribution is then:

m. d. (calculated from  $\overline{11}$ ).  
m. d. (calculated from  $\overline{11}$ ).

-R	.58	<u>I</u>	.67
-S	.25	<u>II</u>	.26
+R	.15	<u>III</u>	.16
+S.	.022	<u>IV</u>	
1.00			109

cf 80 as previous estimates.

This gives a total for the V<sub>1</sub> segregation of 73% R; or 25% crossing over in segregant III which agrees

140. -- + R ++

very well with preceding data  
(v. thesis table 6) giving 27%.

108 -- + S --

Estimating x from these data:

$$\begin{aligned}
 \text{Touch}^2 a &= .022 \times .15 / .58 \times .25 = .0238 & \sqrt{154} & a \\
 " b &= .022 \times .58 / .15 \times .25 = .340 & .583 & .67 \\
 " c &= .022 \times .25 / .15 \times .58 = .064 & .253 & .26 \\
 && \hline & 1.08
 \end{aligned}$$

May 17, 1948.

1. 108 x Y40 On Lac(-) and on Gna EMS'
2. W-67 X Y46 On Lac
3. W-126 X Y40. On Lac

I: gna: Yield < 10/plate. Test on glucose EMS: T1.

$-R$	$\rightarrow$	$+R$	$+S$
22	8	1	1
			32.

I: Lac.	-	+	Lac +: 6 <sup>R</sup> : 1 <sup>S</sup>	all Lac +
9	9	0		
20	20	0		
24	24	1	Lac -	All Lac -
1	1	0		
5	5	0		
4	4	0		
2	2	1		
13	13	1		
4	4	1		
3	3	0		
3	3	0		
10	10	2		
8	8	0		
6	6	0		
6	6	1		
4	4	0		
3	3	0		
3	3	0		
2	2	0		
130	7		137.	5.1% -

The distribution is:

$-R$	$-S$	$+R$	$+S$
.684	.275	.044	.007

Total  $V^R$  segregants:

28.2% ~~E.S.~~

(2).

+ 3	- 0
- 1	0 0
- 1	0 0
- 2	0 0
- 1	0 0
- 1	0 0
- 3	0 0
- 3	0 0
- 1	0 0
- 4	0 0
- 4	0 0
- 1	0 0
- 3	0 0
- 2	0 0
- 1	0 0
<u>32</u>	<u>0.</u>

W-67 x Y-46.

B-M-Lacy - X T-L-B, - V,<sup>R</sup>.

R S

29 2 | 31.

(3).

Lac-	R	S	-R	,47
	11	4	+R	,27
	22	10	-S	,22
	732		+S.	,04
+ 1	2			
- 0	3			
- 7	13			
- 6				
- 1	0			
- 10	3			
- 2	6			
- 2	15			
- 1	0			
- 4	3			
- 1	6			
- 16	33			
- 2	9			
- 1	8			
<u>- 54</u>	<u>118</u>	<u>172</u>		

Lac +      \*      8      3      +> rather high,  
               19      0      otherwise agrees with  
               20      3      sign. of Lac - 1.

47      6      153

31%+ (Maybe excessive)

On these plates, - colonies were much smaller than + possibly distorting ratios.

W-108 X Y-40.

p/187:  $17\text{f} : 254-$  on lactose. ie  $6.7\% \text{ Lac}_3\text{f}$ Among  $\text{f}$ ,  $6 V_1^R : 2 V_1^S$ . $- \quad 99 \quad : \quad 25 \quad \quad \quad 80\% R.$ /191:  $56\text{f} : 463-$  i.e.  $13\% \text{ Lac}_3\text{f}$ For agreement of  
Lac - segregations,

$$\chi^2_2 = 22.2$$

$$p = < .001$$

/198:  $105\text{f} : 512-$   $17\% \text{ Lac}-$ Among  $\text{f}$ ,  $52\text{R}:8\text{S}$   $13\% S.$ Among -  $56\text{R}:24\text{S}$ (70% R) among Lac-.  $\rightarrow$  cf 187.

$$\chi^2_1 = 2.83$$

$$p = .09$$

for fit of V.R.

199. ~~130~~  $: 130 - : 7 +$   $5.1\% \text{ Lac}_3+$ Among +  $6\text{R} : 1\text{S}$ Among -  $82\text{R} : 33\text{S}$ 

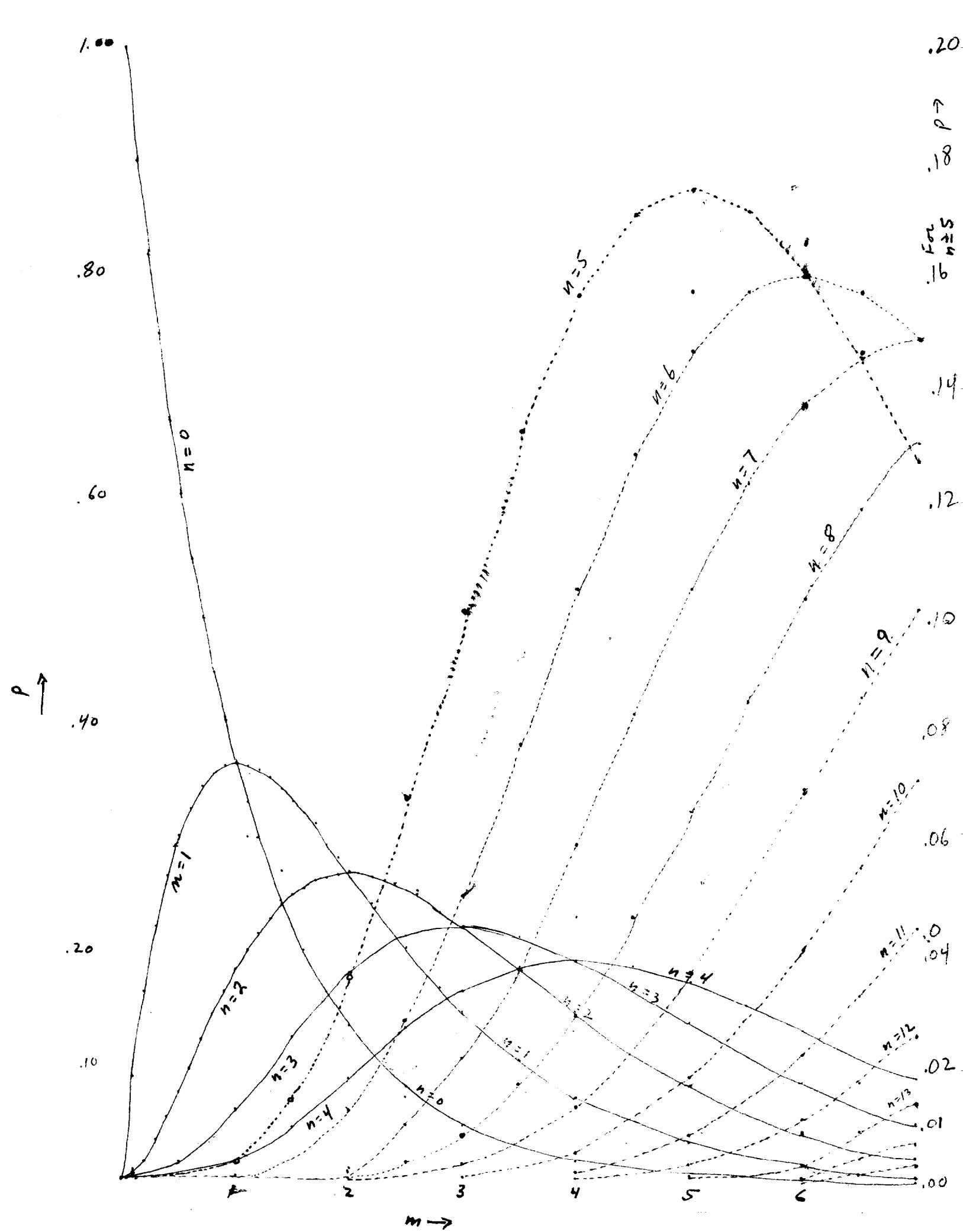
(71% R.)

199 (transf. from galactonate E.H.S.).

	$R$	$S$	
-	30	22	8
+	2.	1	1

$$= 73\% R.$$

All agree on Lac- = Gal-  
Lac+ = Gal+  
on total test for R.  
 $= 344 / 512 = 68\%$



fermentation + hydrolysis

	coli	coli	coli	Aerobacter	Salic.	Typh.
Maltose	+			+	+	+
Saccharose	+			+	-	-
Melibiose	+					
Gentiobiose						
Cellobiose	+		some -			
sucrose	±			+	-	
Fruktose	+					
Raffinose	±					
Salicin	±			+	+	
Anhydrolin	+			+	-	

C<sub>2</sub>+C<sub>3</sub>

(Compound) E.coli E.coli E.coli Aerobacter Salmonella E.typhii

Glyceraldehyde + + +

Dihydroxyacetone + +

Glycerol + - + - +

$\text{CH}_3-\underset{\text{O}}{\text{CH}}-\text{CH}_2$  - -

$\text{CH}_3-\text{CH(OH)}-\text{CH}_2\text{OH}$  +

$\text{H}_2\overset{\text{O}}{\underset{\text{C}}{\text{C}}}=\text{CH}_2$  - -

$\text{HOH}_2\text{C}-\text{CH}_2\text{OH}$  - +

*E. coli* *coli* *coli* *Aerobacter* *salmonicella* *C. typhi*

erythritol - - - - -

Adonitol - +

K-12

	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>Aerobac</i>	<i>Selen</i>	<i>Typh</i>	<u><i>plis</i></u>
D-Arabinoose	+			+	+	-	
L-Arabinoose	+	+		+	+	-	
D-Ribose	+			+	+	+	+
L-Ribose	+			+	+	-	
D-Lyxose	+			+	+	-	
D-Xylose	+	+		+	+	-	
L-Rhamnose	+	K-12: -		+	+	-	
arabinose ac.	-	+		-	+	-	+
xylose ac.	+			+	+		
$\alpha$ -methylarabinose	-			+	-	-	
$\beta$ -methyl xylose	-			-	-	-	
$\alpha$ -methylmannose	-			-			
D-erabitol	-			+		-	

	<u>C<sub>6</sub> + deoxy.</u>					
	<del>K-12</del>	K-12	<i>Acetobacter</i>	<i>Salmonella</i>	<i>Typhi</i>	(continued from previous page)
glucose	+		+	+	+	+
mannose	+		+	+	+	+
galactose	+		+	+	+	-
sorbitol	+		+	+	+	+
dulcitol	±		-	-	±	±
inositol	-	+		+	-	-
mannitol	+		+	+	+	+

d-glucuronic	+			+	+	+
L-galacturonic	+			-	+	-
muconic	±			±	±	-
d-saccharic	+			+	±	-
glucosaminic	+			+	+	-
d-mannuronic	+			+	-	-
glycuronic	+			+	+	-
d-methyl glucoside	-	+	chiral + occ. form.	+	-	-
see over.						
B-methyl glucoside	+ ✓	+		+ -	+	
d-methyl galactoside	+ -	+	-	+ -	?	
B-methyl galact.	+ ✓	+	-	+ -	.	
tetramethyl glucoside	-	.		-		
3-methyl glucose	-			-	-	
d-methyl mannoside	-			-	-	
B-methyl fructoside	-			-	-	

$\alpha \phi$ glucoside	<i>coli</i>
$\beta \phi$ glucoside	-
$\alpha \phi$ galactoside	-
$\beta \phi$ galactoside	+ (Lectose adap.)